THE PRODUCTION OF ANTIBIOTICS IN SOILS

BY

PAUL SIMINOFF

B.S., Michigan State College, 1948
M.S., University of Illinois, 1949

AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN AGRONOMY
IN THE GRADUATE COLLEGE OF THE
UNIVERSITY OF ILLINOIS, 1951

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Introduction

The investigations reported deal with the effects of the production of antibiotics on the biodynamics of the soil. This information is pertinent to such problems as control of soil-borne plant diseases, the introduction of *Azotobacter* into soils and inoculation of soils containing the appropriate legume host with the root-nodule bacteria.

Considerable work has been reported in the literature which tends to show that many of the soil-inhabiting micro-organisms are capable of producing antibiotic substances on artificial media. It has also been shown that soil invaders tend to disappear from soil and a few cases of toxic soil extracts have been reported.

However, the evidence has been contradictory, indicating the necessity for a fundamental approach to the study of antagonistic relationships between microoganisms in the soil. These investigations include biological studies of the interaction in soil between an antagonist and a susceptible organism and physico-chemical studies of the effect of the soil and other natural substrates upon the antibiotic produced by the antagonist.

The antibiotics studied were the basic compounds streptomycin and streptothricin, and the nonbasic substances chloromycetin, actidione and clavacin.

Methods

The interaction between an antagonist and susceptible organism was studied by culturing the two organisms in sterile soil and comparing the growth of each when cultured alone and in mixed cultures.

To study the adsorption of the antibiotic by natural substrates such as illite and bentonite clays and various soil types,

an aqueous solution of the antibiotic was shaken with the substrate, the suspension centrifuged and the amount of antibiotic remaining in the supernatant fluid determined by assay against a sensitive microorganism seeded into agar plates.

The activity of the antibiotic in soil was determined by adding various amounts to soil, allowing the treated soils to stand for 24 hours and then inoculating with a small amount of a susceptible organism. After an incubation period, usually 72 hours, the soils were plated and the numbers of the susceptible organism estimated at the various concentrations of the antibiotic.

The effect of the soil microflora on the stability of chloromycetin and clavacin was determined by adding the antibiotics to both sterile and nonsterile soil and comparing the stability of the two antibiotics under these soil conditions. Approximately 70 per cent of the chloromycetin added could be recovered by extracting the moist soil with methanol while 30 per cent of the clavacin was recoverable by extraction with 10 per cent ethanol buffered at pH 6.2.

Conclusions

Both a streptomycin- and nonstreptomycin-producing strain of *Streptomyces griseus* inhibited the growth of *Bacillus subtilis* in soil. The presence of streptomycin in the soil could not be detected by extraction with water, HCl at pH 2 or acid-acetone. Displacement of streptomycin from the soil complex with methylene blue or janus green was substantially unsuccessful.

Soil in which *S. griseus* was grown did not support the growth of a streptomycin-dependent strain of *Escherichia coli.*

Both streptomycin and streptothricin were adsorbed by clays. One mg. of illite adsorbed 14.5 units of streptomycin and 9.4 units of streptothricin. One mg. of bentonite adsorbed 80 units of streptomycin and 50 units of streptothricin. The adsorption mechanisms were further elucidated by x-ray

diffraction studies of flocculated bentonite.

Streptomycin and streptothricin were rapidly adsorbed and inactivated by soil. At concentrations as great as 500 units per gm., neither antibiotic was able to inhibit the growth of *B. subtilis* in soils.

A chloromycetin-producing strain of *Streptomyces venezuelae* failed to inhibit the growth of *B. subtilis* in soil, although approximately 0.5 μg. of chloromycetin per gm. was detected in methanol extracts of soil cultures of the actinomycete. The production of chloromycetin could be increased to about 25 μg. per gm. of soil by the addition of 1 per cent tryptone and to about 1.5 μg. per gm. by addition of 1 per cent green alfalfa hay. No production could be detected with oat straw or starch.

A clavacin-producting strain of *Aspergillus clavatus* inhibited the growth of *B. subtilis* in soil but this antagonism was not associated with the production of clavacin in the soil cultures.

Chloromycetin, actidione and clavacin were active in the soil against susceptible microorganisms, although the activity levels were lower than those obtained in nutrient broth. Fifteen μg. of chloromycetin per gm. completely inhibited the growth of *B. subtilis* in soil. The growth of *Saccharomyces pastorianus* in glucose-amended soil was inhibited by 5 μg. of actidione per gm. of soil. Between 20 and 40 μg. per gm. of clavacin inhibited *B. subtilis* in soil.

The adsorption of the nonbasic antibiotics by natural substrates was relatively low and all could be recovered from soil by ordinary extraction procedures involving shaking the treated soils with water or appropriate organic solvents.

Both chloromycetin and clavacin were rapidly degraded in nonsterile soils, while remaining stable in sterile soils. Of the initial treatment with 50 μg. of antibiotic per gm. of soil, only 5 μg. of chloromycetin were recovered at the end of two weeks, while less than 5 μg. of clavacin could be detected

after two days. The breakdown of clavacin was associated with the development of a predominantly clavacin-resistant soil microflora.

It is concluded that basic antibiotics such as streptomycin and streptothricin do not affect the biological equilibria of the soil due to rapid adsorption and inactivation by the soil complex. Nonbasic antibiotics such as chloromycetin and clavacin, while potentially active in the soil, are not major factors in the biodynamics of the soil due to low levels of production under ordinary soil conditions and to rapid degradation by the soil microflora.

VITA

The author was born May 8, 1923 in Brooklyn, New York. He received his primary and secondary school education in New York City. His college education was begun in Brooklyn College in 1940 but was interrupted by service in the army during the years 1942 to 1945. Upon discharge from the army, the author resumed undergraduate work at Michigan State College from which he received a Bachelor of Science degree with honors in Soil Science in June, 1948. He entered the graduate school of the University of Illinois in September, 1948 and received the degree of Master of Science in Agronomy in August, 1949. He held a Wright Fellowship during his second year of graduate study and a University Fellowship during his third year.

The author is a member of Sigma Xi, Phi Kappa Phi, Alpha Zeta, and Gamma Sigma Delta.

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